Genotypic polymorphism of *Spodoptera litura* nucleopolyhedrovirus (SpltNPV) in the wild

LIU Yan-He[1,2], WANG Li-Hua[1], FANG Ji-Chao[1,*]

(1. Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China;
2. Agricultural College, Guangdong Ocean University, Zhanjiang, Guangdong 524088, China)

Abstract: 【Aim】 This study aims to further reveal genotypic polymorphism of *Spodoptera litura* nucleopolyhedrovirus (SpltNPV) in the wild based on genome sequences. 【Methods】 The virus clones A_5, F_1, X_3 and X_{15} were isolated from SpltNPV Egypt stock, Fukuyama stock and Ogasawara stock by *in vivo* cloning technique, respectively. According to the complete genome sequence of SpltNPV (GenBank accession no.: NC_003102) and some SpliNPV gene sequences (GenBank accession no.: X99377, X99376 and X98924), primers were designed. *Polyhedrin* and ORF18 – ORF23 of A_5, F_1, X_3 and X_{15} were obtained by PCR amplification. 【Results】 According to the *polyhedrin* sequence, A_5 and F_1 belong to SpliNPV type, while X_3 and X_{15} belong to SpltNPV type. The homologous comparisons of ORF18 – ORF23 were done between A_5, F_1, X_3, X_{15} and SpltNPV or SpliNPV. The results showed that F_1 shares high nucleotide identity with SpliNPV, and X_3 shares high nucleotide identity with SpltNPV, but only 387 bp is deleted at 172 – 558 nt of ORF20. Though X_{15} belongs to SpltNPV type according to the *polyhedrin* sequence, ORF18 – ORF23 all share high nucleotide identities to those of SpliNPV, and as a SpltNPV-unique ORF, ORF22 is deleted in X_{15} genome. A_5 belongs to SpliNPV type, and the corresponding ORF18 – ORF20 share high nucleotide identities with SpliNPV, but ORF21 shares 100% nucleotide identity with that of SpltNPV. As a SpltNPV-unique ORF, ORF22, however, appears in A_5 genome, and shares 100% nucleotide identity with that of SpltNPV. Furthermore, ORF23 shares high nucleotide identity with that of SpliNPV. 【Conclusion】 These results indicate that SpltNPV in the wild possesses genotypic polymorphisms, and moreover, the genomes of these virus clones with the same genotype have significant differences. The natural heterogeneity can be exploited to develop NPV strains suitable for the control of *S. litura*.

Key words: *Spodoptera litura* nucleopolyhedrovirus (SpltNPV); virus clones; genotype; genome; polymorphism

1 INTRODUCTION

The common cutworm, *Spodoptera litura* (Fabricius), that feeds on over 80 species of plants (Okamoto and Okada, 1968), is one of the most economically important insect pests in many countries including Japan, China, India, and other countries of Southeast Asia. Nucleopolyhedroviruses (NPVs) that infect *S. litura* have been isolated from several Asian locations, and some isolates have been tested for controlling larval populations of *S. litura* and recognized as a potential alternative for the management of this insect. According to *in vitro* host range, polyhedrin (polh) size, peptide mapping and DNA homology studies, *S. litura* NPV (SpltNPV) is separated into four distinct groups: group I, isolates corresponding to *Autographa californica* NPV (AcNPV); groups II and IV, two different groups of isolates of *Spodoptera littoralis* NPV which had been previously characterized as SliNPV-B (or SliNPV-T) and SliNPV-A (or SliNPV-D), respectively (Kislev and Edelman, 1982; Cherry and Summers, 1985; Croizier *et al.*, 1989); and group Ⅲ, isolates not corresponding to any reported virus group (Maeda *et al.*, 1990). Nine Asia isolates of SpltNPV and an Egyptian isolate of SpliNPV could be broadly divided into two groups based on restriction endonuclease (REN) patterns: SpltNPV-type and SpliNPV-type (Takatsuka *et al.*, 2003).

The phylogenetic tree analysis based on nucleotide sequences of the polyhedrin genes indicated that SpltNPV could be broadly divided into three groups: SpliNPV type, SpltNPV type and *S. exigua* NPV (SeNPV) type. SpliNPV type and SpltNPV type are clustered into one clade, but

基金项目：江苏省农业科技创新资金项目[cx(11)2046]；公益性行业科研专项(201203036)
作者简介：刘艳荷，女，1965 年生，河北迁安人，博士，副教授，研究方向为昆虫病毒分子生物学，E-mail：liuyanhe524@yahoo.com.cn
* 通讯作者 Corresponding author, E-mail: fangjc@jaas.ac.cn
收稿日期 Received: 2012-12-23；接受日期 Accepted: 2013-03-11

SeNPV type is far from SpliNPV type and SpltNPV type, and positions within a monophyletic clade together with SeNPV and *S. frugiperda* NPV (SfNPV) (Liu *et al.*, 2011). One hundred eighty-nine clones obtained from the SpltNPV-infected larvae collected in Japan exhibit 33 distinct REN patterns that represent three distinct NPV types, designated as type A, type B and type C. Type A corresponds to SpliNPV-D or SpliNPV-B, type B corresponds to the NPVs widely identified in *S. litura* larvae in Japan, China, and the Philippines (Maeda *et al.*, 1990; Laviña *et al.*, 2001; Laviña-Caoili *et al.*, 2001; Pang *et al.*, 2001; Takatsuka *et al.*, 2003), but type C is different from type A or type B, and is a novel NPV type that is pathogenic to *S. litura* (Kamiya *et al.*, 2004). In the present study, 5 pairs of primers were designed based on SpltNPV genomes and some SpliNPV gene sequences, and *polyhedrin* and ORF18 – ORF23 of four SpltNPV clones were obtained by PCR amplifications. Through homologous comparison of these sequences with those of SpltNPV or SpliNPV, it was found that SpltNPV not only possesses genotype diversity, but also even if in one genotype, the genomes of these virus clones have significant differences. The natural heterogeneity of SpltNPV will provide a theoretical basis for SpltNPV exploitation and utilization.

2 MATERIAL AND METHODS

2.1 Insects

S. litura larvae were collected from cabbage fields in Nanjing, Jiangsu Province, China, and reared in the laboratory at 28 ± 1℃, 50% – 60% RH and a 14L: 10D photoperiod. Larvae were group reared on wheat germ based artificial diet in column-form glass jars (30 cm in diameter, and 20 cm in height) up to the 4th instars, and then individually in six-well tissue culture plates. Pupae were kept in similar glass jars and, on emergence, adults were paired in glass cages (30 cm × 30 cm × 30 cm) and provided with 5% sugar solution as the food source. Paper was provided as the oviposition substrate, and eggs were sterilized with 5% fomaldehyde solution. After hatching, larvae were reared as described above until the desired developmental stages used in the experiments were obtained.

2.2 Virus preparation

SpltNPV isolates were originally collected from diseased larvae of *S. litura* in Japan, and the clones were isolated by *in vivo* cloning technique (Guo, 2005) and kept in Crop Pest Laboratory, Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences. A_5 clone was isolated from Egypt stock, F_1 clone was from Fukuyama stock, X_3 and X_{15} were from Ogasawara stock. All clones had been propagated in *S. litura* larvae. *Polyhedrin* occlusion bodies (POBs) were obtained through feeding early 4th-instar larvae with artificial diet contaminated with the appropriate virus in six-well tissue culture plates. The infected larvae were reared at 29℃, 50% RH and a 14L: 10D photoperiod, and collected after death. To produce sufficient viruses for experiments, POBs were recovered from a pool of infected larvae and purified, first by filtration through cheesecloth to remove larvae debris, and then by centrifugation at 500 r/min for 5 min and at 3 000 r/min for 15 min. The pure POBs were stored at 4℃ until use (Guo *et al.*, 2007a, 2007b).

2.3 Virus DNA extraction

Viral DNA was isolated from approximately 10^9 POBs in 300 mL of water. POB suspensions were treated with a one-third volume of 3 × DAS (0.3 mol/L Na_2CO_3, 0.5 mol/L NaCl, 0.03 mol/L EDTA, pH 10.5) at 37℃ for 5 min to dissolve the *polyhedrin* matrix. Undissolved POBs and other heavy particulate material were pelleted by centrifugation at 8 000 r/min for 8 min. The virion-containing supernatant was transferred to sterile microcentrifuge tubes and incubated with proteinase K (200 mg/mL) at 45 to 50°C for 2.5 h and then with 1% sodium dodecyl sulfate (SDS) for an additional 0.5 h. Viral DNA was extracted once with an equal volume of TE [10 mmol/L Tris, 1 mmol/L EDTA (pH 8.0)] buffer-saturated phenol and twice with equal volumes of TE buffer-saturated phenol-chloroform-isoamyl alcohol (25:24:1, v/v). The DNA suspension was dialyzed against three to four changes of 0.01 × TE at 4℃ for 48 h. The DNA yields ranged between 60 – 90 mg (Muñoz *et al.*, 1997).

2.4 PCR analysis

The polymerase chain reaction (PCR) was used to amplify the virus genes. Primers used in this experiment were in Table 1, and these primers were designed based on the sequences of *polyhedrin* genes (GenBank accession no.: AY600451), SpltNPV genomes (GenBank accession no.: NC_003102) and some SpliNPV genes (GenBank accession no.: X99377, X99376 and X98924) deposited in GenBank. PCR was performed by using 10 μL buffer, 8 μL $MgCl_2$ (25 mmol/L), 8 μL dNTPs mix (2.5 mmol/L each nucleotide), 2.5 U Taq DNA polymerase (Promega), 100 ng each primer, 30 ng template DNA and H_2O to a volume of 50 μL. A

total of 30 reaction cycles were performed with denaturing at 94℃ for 1 min, annealing at 55℃ for 1 min, and extension at 72℃ for 2 min, following a final extension at 72℃ for 10 min. After amplification, 10 μL of the reactions were used to confirm their correct size by agarose gel electrophoresis.

Table 1 PCR primers used in this study

Amplified gene	Primer name	Nucleotide sequence (5′-3′)
polyhedrin	F	ATGTATAGTCGTYATAYAGYGCCTACA
	R	TTARTABGCGGGTCCGTTGTAHAGA
ORF18 - ORF23	P_1	AGGTGATGATTGAATAGGACATGGAA
	P_2	GTCGATCASATTCTAACGTTTCTGC
	P_3	GCAGAAACGTTAGAATSTGATCGAC
	P_4	TGACKTCGATACATTTTTACGCGTG
	P_5	CACGCGTAAAAATGTATCGAMGTCA
	$P_{6\text{-}A}$	TGTTGTCTTCCGACTATGTTACCCAA
	$P_{6\text{-}B}$	TTCTGAACTCATCATGTCTTACGACC

2.5 Gene cloning and sequencing

The amplification products were inserted into pGEM-T easy vector (Promega). The products of the ligation mixture were transformed into competent *Escherichia coli* cells (TG1). Transformed *E. coli* cells were then inoculated in LB agar dishes containing ampicillin, IPTG, and X-gal. Totally 10 white colonies were selected for further analysis. The plasmids from these colonies were extracted and digested with *Eco*R Ⅰ to confirm the correct insertion. Three positive clones for correctly inserted fragment were picked, sequenced using universal primers, *i. e.*, M13/pUC forward and reverse primers synthesized at Sangon Biotech Company (Shanghai, China).

2.6 Data analysis

SpltNPV *polyhedrin* and other gene sequences used in this study were downloaded from GenBank. The obtained sequences were compared with the sequences in the GenBank using the BLAST program (Altschul *et al.*, 1997). Alignment of nucleotide sequences or amino acid sequences was operated using Clustalw2 (http://www.ebi.ac.uk/Tools/msa/clustalw2).

3 RUSULTS

3.1 Homologous analysis of *polyhedrin*

Polyhedrin genes of the virus clones A_5, F_1, X_3, and X_{15} were amplified by PCR using *polyhedrin*-specific primers F and R. The results manifested that *polyhedrin* genes from four clones are all 750 bp in length and encode a peptide of 249 amino acids. Alignment of *polyhedrin* genes and their homologues showed that the nucleotide sequence of A_5-*polh* shares 99% identity with that of SpliNPV-*polh* (GenBank accession no.: AY600451) with 5 necleotides different, which are located at 12 and 15 nt (c-t), 726 nt (a-t), 729 nt (c-a), and 733 nt (a-g), respectively, correspondingly only 1 aa different, located at 733 nt (T-A). F_1-*polh* also shares 99% nucleotide sequence identity with that of SpliNPV-*polh*, 3 different nucleotides are located at 12 and 15 nt (c-t), and 729 nt (c-a), respectively, but no amino acid sequence difference was found. X_3-*polh* share 99% nucleotide sequence identity with that of SpltNPV-*polh* with only 3 nucleotides different, located at 90 nt (c-t), 735 nt (g-c), and 747 nt (t-c), respectively, X_{15}-*polh* share 99% nucleotide sequence identity with that of SpltNPV-*polh* with only 1 nucleotide different, located at 747 nt (t-c), and the encoded amino acid sequences are all the same. All these results based on *polyhedrin* sequences analysis indicate that X_3, X_{15} are the members of SpltNPV genotype, while A_5 and F_1 belong to SpliNPV genotype.

3.2 Cloning of ORF18 - ORF23 and homologous comparison

By using primers P_1 and P_2, P_3 and P_4, P_5 and $P_{6\text{-}A}$, and P_5 and $P_{6\text{-}B}$, respectively, 12 fragments about 2 000 bp in size were amplified from A_5, F_1, X_3, and X_{15} genomic DNA (Fig. 1). The fragments were cloned, sequenced and assembled, finally the four sequences about 6 000 bp in length were obtained, including ORF18, ORF19 (*p*10), ORF20, ORF21 (074), ORF22 and ORF23 (*rr*1), with the GenBank accession numbers of GU446712, GU446713, GU446714 and GU446715, respectively.

Alignment of ORF18 - ORF23 and its homologues showed that X_3 shared high identity with SpltNPV, ORF18, ORF19, ORF21, ORF22 and ORF23 share 99% identities with the homologues of SpltNPV, only 387 bp is deleted at 172 - 558 nt of ORF20. F_1 and X_{15} share high identities with SpliNPV, their ORF18 are completely identical with that of SpliNPV, and their other ORFs share 99% identities with those of SpliNPV. The most interesting was A_5 clone, its ORF18, ORF19 and ORF20 share 100%, 99% and 99% identities with that of SpliNPV, and 91%, 91% and 85% identities with that of SpltNPV; its ORF21 shares 99% identity with that of SpltNPV, and only 90% identity with that of SpliNPV; ORF22 is unique to

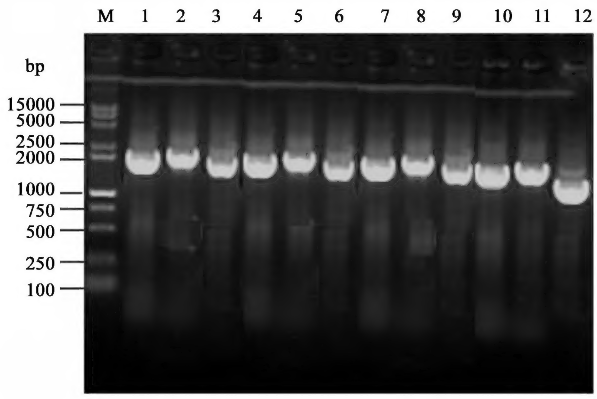

Fig. 1 PCR products of several SpltNPV clones by agarose gel electrophoresis

M: DNA marker DL2000 + 15000; 1 – 3: PCR products amplified with primers P_5 and $P_{6\text{-}A}$, P_3 and P_4, and P_1 and P_2 from A_5, respectively; 4 – 6: PCR products amplified with primers P_5 and $P_{6\text{-}A}$, P_3 and P_4, and P_1 and P_2 from F_1, respectively; 7 – 9: PCR products amplified with primers P_5 and $P_{6\text{-}A}$, P_3 and P_4, and P_1 and P_2 from X_{15}, respectively; 10 – 12: PCR products amplified with primers P_5 and $P_{6\text{-}B}$, P_3 and P_4, and P_1 and P_2 from X_3, respectively.

SpltNPV and deleted in SpliNPV, and ORF22 of A_5 clone shares 100% identity with that of SpltNPV. Interestingly, ORF23 of A_5 clone shares 97% identity with that of SpliNPV, while 88% identity with that of SpltNPV (Table 2).

3.3 Genotypic polymorphism of SpltNPV

According to *polyhedrin* sequence, F_1 belongs to SpliNPV type. As for the region of ORF18 – ORF23, the identities between F_1 and SpliNPV are all higher than those between SpltNPV and F_1. X_3 belongs to SpltNPV type and the corresponding identities of X_3 and SpltNPV are all higher than those of X_3 and SpliNPV, but 387 bp is deleted at 172 – 558 nt of ORF20. Interestingly, although X_{15} belongs to SpltNPV type, in ORF18 – ORF23 region, the identities of X_{15} and SpltNPV are lower than those of X_{15} and SpliNPV. Above all, as a SpltNPV unique ORF, ORF22 is deleted in X_{15} genome. Similarly, A_5 belongs to SpliNPV type, and the corresponding identities in ORF18 – ORF20 region between A_5 and SpliNPV are higher than those between A_5 and SpltNPV, but the identities of ORF21 – ORF22 between A_5 and SpliNPV are lower than that between A_5 and SpltNPV. The identity of ORF23 between A_5 and SpliNPV is higher than that between A_5 and SpltNPV. ORF22 only exists in SpltNPV genome and lost in SpliNPV genome, but it appears in A_5 genome. ORF22 in A_5 genome shares 100% identity with that in SpltNPV genome (Fig. 2). All these results suggested that SpltNPV in the wild possess genotypic polymorphisms, and moreover, the genomes of these virus clones with the same genotype have significant differences.

Table 2 Identities of nucleotide and amino acid sequence of ORF18 – ORF23 between some clones and SpltNPV or SpliNPV

nt (%) / aa (%)	ORF18						ORF19						ORF20					
	SptN	A_5	F_1	X_3	X_{15}	SpiN	SptN	A_5	F_1	X_3	X_{15}	SpiN	SptN	A_5	F_1	X_3	X_{15}	SpiN
SptN		91	91	99	91	91		91	90	99	91	90		84	84	99	84	84
A_5	95		100	91	100	100	96		99	90	99	99	83		100	87	100	99
F_1	95	100		91	100	100	90	99		90	99	99	83	100		87	100	99
X_3	100	95	95		91	91	99	95	89		90	90	98	86	86		87	87
X_{15}	95	100	100	95		100	96	100	99	95		99	83	100	100	86		99
SpiN	95	100	100	95	100		96	100	99	95	100		82	99	99	85	99	

nt (%) / aa (%)	ORF21						ORF22						ORF23					
	SptN	A_5	F_1	X_3	X_{15}	SpiN	SptN	A_5	F_1	X_3	X_{15}	SpiN	SptN	A_5	F_1	X_3	X_{15}	SpiN
SptN		99	90	99	90	89		100	×	99	×	×		88	87	99	86	86
A_5	99		91	98	91	90	100		×	99	×	×	88		98	88	97	97
F_1	92	93		90	99	99	×	×		×	×	×	87	98		87	99	99
X_3	99	99	93		90	89	99	99	×		×	×	99	88	87		86	86
X_{15}	92	93	99	92		99	×	×	×	×		×	87	98	99	87		99
SpiN	89	89	95	89	95		×	×	×	×	×		85	96	97	85	97	

SptN: SpltNPV; SpiN: SpliNPV; nt: Nucleotide; aa: Amino acid.

4 DISCUSSION

SpltNPV is the member of Baculoviridae, alphabaculovirus (King *et al.*, 2011). It has a variety of stocks in the wild based on the restriction

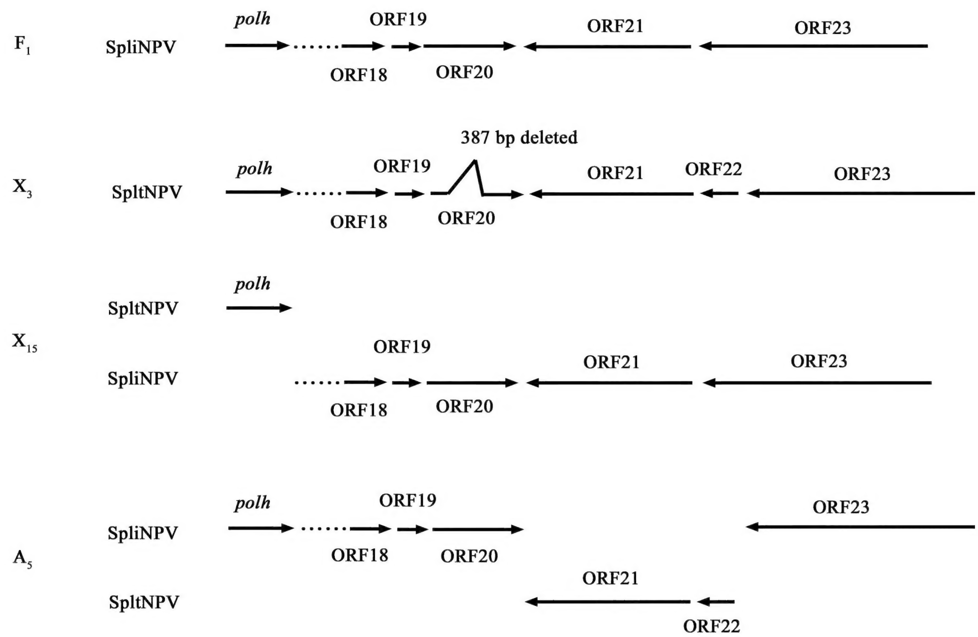

Fig. 2 Genome diversities of SpltNPV A_5, F_1, X_3, and X_{15} clones
polh: *polyhedrin*. The dots indicate the omitted ORFs, and the arrows show the gene encoding direction.

endonuclease (REN) patterns of viral genomic DNA. Even in the stocks collected within the same district, there were still some submolar bands to some extent in REN patterns of viral genomic DNA, suggesting that the wild virus stocks contain many isolates, some of which may even belong to different virus species. Of nine SpltNPV isolates collected from Asia and an Egyptian isolate of *S. littoralis* NPV, the submolar bands in REN analysis were detected in 6 isolates (Takatsuka *et al.*, 2003). The Ogasawara stock contained representatives of three virus groups (Ⅰ, Ⅱ and Ⅲ) and the Chikugo stock contained two different groups (Ⅲ and Ⅳ) (Maeda *et al.*, 1990). Kamiya *et al.* (2004) isolated A and B genotypes from the 7th stock AI-A-S, and B and C genotypes from the 17th stock GI-G3-F. We also isolated SeNPV, SpliNPV and SpltNPV genotypes from the Ogasawara stock (Liu *et al.*, 2011). The existence of these different viruses in the wild stocks may assist in the propagation of progeny viruses when the original virus stock is ingested by different insect hosts.

The homologous recombination of intra-specific and inter-specific in Baculoviruses occurred after *per oral* infection host larvae, and result in the generation of a new recombinant virus (Muñoz *et al.*, 1997; Jehle *et al.*, 2003). According to the previous study, SpltNPVs were classified into three distinct types, designed as type A (SpliNPV), type B (SpltNPV) and typed C (SeNPV), respectively (Kamiya *et al.*, 2004; Liu *et al.*, 2011). The homologies between type A and type B were higher with 94% – 95% *polyhedrin* nucleotide sequence identities and 98% – 100% amino acid sequence identities. In contrast, the homologies between type C and type A or type B were lower, only 76% – 78% *polyhedrin* nucleotide sequence identities and 81% – 82% amino acid sequence identities (Liu, 2010). This study has proven that the homologous recombination may happen between SpltMNPV and SpliNPV in the wild, and correspondingly generate new viruses. However, whether the homologous recombination occurs between type C and type A or type C and type B need further study.

SpltNPVs from the wild possess not only genotypic diversity but also phenotypic diversity. Comparative characterization with *S. litura* cultured cells and larvae showed that the biological properties with respect to productivity and virulence differed significantly not only among the types of SpltNPV but also among the variants within a SpltNPV type, demonstrating that SpltNPV-C caused rapid mortality of *S. litura* larvae compared with those clones of type A or type B SpltNPV (Kamiya *et al.*, 2004; Zhu *et al.*, 2004). Droplet feeding bioassays of virus clones isolated by *in vivo* cloning technique against the 3rd instar larvae of *S. litura* showed significantly different biological activity based on the infectivity and survival time of infected larvae (Guo, 2005), suggesting that the genotypic diversity plays an important role in retaining progeny viruses under different environmental conditions, and we can exploit and utilize the natural heterogeneity of baculoviruses to develop NPV strains suitable for the control of *S. litura*.

References

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ, 1997. A new generation of protein database search programs. *Nucleic Acids Res.*, 25: 3389 – 3402.

Cherry CL, Summers MD, 1985. Genotypic variation among wild isolates of two nuclear polyhedrosis viruses isolated from *Spodoptera littoralis*. *J. Invertebr. Pathol*., 46: 289 - 295.

Croizier G, Boukhoudmi-Amiri K, Croizler L, 1989. A physical map of *Spodoptera littoralis* B-type nuclear polyhedrosis virus genome. *Archives of Virology*, 104: 145 - 151.

Guo HF, 2005. Approaches and Mechanisms for Improvement of Nucleopolyhedroviruses. PhD Dissertation, Nanjing Agricultural University, Nanjing.［郭慧芳, 2005. 昆虫核型多角体病毒的增效途径和机理. 南京: 南京农业大学博士学位论文］

Guo HF, Fang JC, Liu BS, Wang JP, Zhong WF, Wan FH, 2007a. Enhancement of the biological activity of nucleopolyhedrovirus through disruption of the peritrophic matrix of insect larvae by chlorfluazuron. *Pest Management Science*, 63(1): 68 - 74.

Guo HF, Fang JC, Wang JP, Zhong WF, Liu BS, 2007b. Interaction of *Xestia c-nigrum* granulovirus with peritrophic matrix and *Spodoptera litura* nucleopolyhedrovirus in *Spodoptera litura*. *Journal of Economic Entomology*, 100(1): 20 - 25.

Jehle1 JA, Fritsch E, Huber J, Backhaus H, 2003. Intra-specific and inter-specific recombination of tortricid-specific granuloviruses during co-infection in insect larvae. *Arch. Virol*., 148: 1317 - 1333.

Kamiya K, Zhu J, Murata M, Laviñña-Caoili BA, Ikeda M, Kobayashi M, Kawamura S, 2004. Cloning and comparative characterization of three distinct nucleopolyhedroviruses isolated from the cotton cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae) in Japan. *Biological Control*, 31(1): 38 - 48.

King AMQ, Lefkowitz E, Adams MJ, Carstens EB, 2011. Virus Taxonomy. Ninth Report of the ICTV. Academic Press/Elsevier, San Diego.

Kislev N, Edelman M, 1982. DNA restriction pattern differences from geographic isolates of *Spodoptera littoralis* nuclear polyhedrosis virus. *Virology*, 119: 219 - 222.

Laviña BA, Padua LE, Wu FQ, Shirata N, Ikeda M, Kobayashi M, 2001. Biological characterization of a nucleopolyhedrovirus of *Spodoptera litura* (Lepidoptera: Noctuidae) isolated from the Philippines. *Biological Control*, 20: 39 - 47.

Laviña-Caoili BA, Kamiya K, Kawamura S, Ikeda M, Kobayashi M, 2001. Comparative *in vitro* analysis of geographic variants of nucleopolyhedrovirus of *Spodoptera litura* isolated from China and the Philippines. *J. Insect Biotechnol. Sericol*., 70: 199 - 209.

Liu YH, 2010. Genetic difference of *Spodoptera litura* nucleopolyhedrovirus genotypes and chitinase synergistic effect on virus and construction of recombined virus insecticide. Working Report, Jiangsu Academy of Agricultural Sciences Work Station, Jiangsu Academy of Agricultural Sciences Post-doctoral Working Station, Nanjing.［刘艳荷, 2010. 斜纹夜蛾核型多角体病毒基因型遗传差异、几丁质酶对病毒增效作用和重组病毒杀虫剂的研究. 南京: 江苏省农业科学院科研工作站博士后出站报告］

Liu YH, Guo HF, Fang JC, 2011. Sequence analysis of some genes in X_{17} strain of *Spodoptera litura* multinucleocapsid nucleopolyhedrovirus C genotype. *Acta Entomologica Sinica*, 54 (9): 1010 - 1017.［刘艳荷, 郭慧芳, 方继朝, 2011. C 型斜纹夜蛾核型多角体病毒 X_{17}病毒株部分基因的序列分析. 昆虫学报, 54(9): 1010 - 1017］

Maeda S, Mukohara Y, Kondo A, 1990. Characteristically distinct isolates of the nuclear polyhedrosis virus from *Spodoptera litura*. *J. Gen. Virol*., 71: 2631 - 2639.

Muñoz D, Vlak JM, Caballero P, 1997. *In vivo* recombination between two strains of the genus *Nucleopolyhedrovirus* in its natural host, *Spodoptera exigua*. *Applied and Environmental Microbiology*, 63 (8): 3025 - 3031.

Okamoto O, Okada T, 1968. Studies on the tobacco cutworm, *Prodenia litura* Fabricius as an insect pest of forage crops. *Bull. Cyugoku Natl. Agr. Exp. Stn*., E2: 111 - 144.

Pang Y, Yu J, Wang L, Hu X, Bao W, Li G, Chen C, Han H, Hu S, Yang H, 2001. Sequence analysis of the *Spodoptera litura* multicapsid nucleopolyhedrovirus genome. *Virology*, 287 (2): 391 - 404.

Takatsuka J, Okuno S, Nakai M, Kunimi Y, 2003. Genetic and biological comparisons of ten geographic isolates of a nucleopolyhedrovirus that infects *Spodoptera litura* (Lepidoptera: Noctuidae). *Biological Control*, 26: 32 - 39.

Zhu J, Shen SD, Wang WB, Zhu YF, Ikeda M, Hu ZL, Sheng Y, 2004. Proliferation characteristics of three Japanese strains of *Spodoptera litura* multicapsid nucleopolyhedrovirus in cultural cells and sequence analysis of their polyhedrin genes. *Acta Entomologica Sinica*, 7(5): 543 - 550.［朱江, 沈颂东, 王文兵, 朱玉芳, 池田素子, 胡兆丽, 盛晔, 2004. 日本三株斜纹夜蛾核型多角体病毒的增殖特性及其多角体蛋白基因的序列分析. 昆虫学报, 7(5): 543 - 550］

自然界斜纹夜蛾核型多角体病毒的基因型多态性

刘艳荷[1,2]，王利华[1]，方继朝[1,*]

(1. 江苏省农业科学院植物保护研究所，南京 210014；2. 广东海洋大学农学院，广东湛江 524088)

摘要：【目的】从基因组序列角度进一步揭示自然界斜纹夜蛾核型多角体病毒（*Spodoptera litura* nucleopolyhedrovirus，SpltNPV）的基因型多态性。【方法】病毒克隆 A_5，F_1，X_3 和 X_{15} 分别以活体克隆法分离自 SpltNPV 埃及株、日本福冈株和日本小笠原株。根据 SpltNPV 基因组全序列（GenBank 登录号：AF325155）和海灰翅夜蛾核型多角体病毒（*S. littoralis* NPV，SpliNPV）部分基因序列（GenBank 登录号：X99377，X99376 和 X98924）设计引物，PCR 扩增获得 A_5，F_1，X_3 和 X_{15} 的多角体蛋白（polyhedrin，polh）基因和 ORF18～ORF23 序列。【结果】根据多角体蛋白基因序列，X_3 和 X_{15} 属于 SpltNPV 型，而 A_5 和 F_1 属于 SpliNPV 型。将 A_5，F_1，X_3 和 X_{15} 的 ORF18～ORF23 与 SpltNPV 和 SpliNPV 相应的基因序列进行同源性比较。结果发现，F_1 与 SpliNPV 以及 X_3 与 SpltNPV 的核苷酸序列相似性高，但 X_3 的 ORF20 在 172～558 nt 处缺失 387 bp。尽管依据多角体蛋白基因序列 X_{15} 属于 SpltNPV 型，但对于 ORF18～ORF23 序列，X_{15} 与 SpliNPV 的相似性高于与 SpltNPV 的相似性。同样，A_5 属于 SpliNPV 型，ORF18～ORF20 与 SpliNPV 相应的核苷酸序列相似性高，但 ORF21 与 SpltNPV 相应的核苷酸序列一致性为 100%，特别是 ORF22，SpltNPV 的特有序列出现在 A_5 的基因组中，而且与 SpltNPV 的 ORF22 一致性为 100%；反过来，ORF23 又与 SpliNPV 相应的核苷酸序列相似性高。【结论】所有这些都表明，SpltNPV 在自然界不仅存在基因型多态性，而且即使属于同一基因型，它们的基因组序列也有显著差异。可利用 SpltNPV 在自然界的这种异质性筛选适宜防治斜纹夜蛾幼虫的株系。

关键词：斜纹夜蛾核型多角体病毒；病毒克隆；基因型；基因组；多态性

中图分类号：Q965.8 **文献标志码：**A **文章编号：**0454-6296(2013)04-0372-07

（责任编辑：赵利辉）